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Persistence of Exotic Newcastle Disease Virus (ENDV) in Laboratory Infected *Musca domestica* and *Fannia canicularis*

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SUMMARY. House flies (*Musca domestica*) and little house flies (*Fannia canicularis*) were examined for their ability to take up and harbor a velogenic strain of exotic Newcastle disease virus (ENDV) (family *Paramyxoviridae*, genus *Avulavirus*). Laboratory-reared flies were allowed to feed on evaporated milk containing ENDV at a virus concentration of $10^{8.3}$ egg infectious dose $(EID)_{50}$/0.1 ml or on poultry feces containing an ENDV titer of $10^{5.8}$ EID_{50}/0.1 g. Flies exposed to either infectious food source for 24 hr became transiently infected with virus. Virus persisted predominantly in the mid- and hindgut, with relatively little virus isolated from the remainder of the fly body. Virus persisted similarly in both fly species that were fed evaporated milk containing ENDV, with a maximum ENDV titer of $10^{5.98}$ EID_{50}/fly for the house fly and $10^{4.78}$ EID_{50}/fly for the little house fly at 1 day postexposure; titers decreased on subsequent days to $10^{2.38}$ EID_{50}/fly for house fly and $\geq$1 EID_{50}/fly for little house fly at 5 days postexposure. Both fly species acquired viral titers greater than the infective dose for a susceptible chicken ($10^{3.0}$ EID_{50}–$10^{4.0}$ EID_{50}). In addition, flies fed evaporated milk containing a high titer of ENDV maintained viral titers above the infective dose for up to 4 days postexposure to the infectious food source. Flies fed on infective feces retained a chicken infective dose for only one day. The decrease in viral titer over time was significantly explained by logistic regression for both fly species ($P < 0.05$). The slope of the regression line was not different for the two fly species ($P < 0.05$), indicating a similar rate of virus loss.

RESUMEN. Persistencia del virus velogénico de la enfermedad de Newcastle en moscas domésticas (*Musca domestica*) y moscas domésticas pequeñas (*Fannia canicularis*) infectadas experimentalmente.

Se evaluó la capacidad de moscas domésticas (*Musca domestica*) y moscas domésticas pequeñas (*Fannia canicularis*) de infectarse y ser portadores de una cepa velogénica del virus de la enfermedad de Newcastle (familia *Paramyxoviridae*, género *Avulavirus*). Se permitió que moscas criadas en el laboratorio se alimentaran de leche evaporada contaminada con virus velogénico de la enfermedad de Newcastle a una concentración de $10^{8.3}$ dosis infectiva 50 para embrión por cada 0.1 ml (DIE_{50}/0.1 ml) o en heces de aves que contenían virus velogénico de la enfermedad de Newcastle a una concentración de $10^{5.8}$ DIE_{50}/0.1g. Las moscas expuestas por 24 horas a cualquiera de las dos fuentes de alimentación se infectaron transitoriamente con el virus. El virus persistió predominantemente en la porción media y distal del intestino, con relativamente poco virus aislado del resto del cuerpo de las moscas. El virus persistió de manera similar en las dos especies de moscas que fueron alimentadas con la leche evaporada que contenía el virus velogénico de la enfermedad de Newcastle, mostrando un día posterior a la infección un título máximo de $10^{5.9}$ DIE_{50}/mosca para la mosca doméstica y de $10^{4.7}$ DIE_{50}/mosca para la mosca doméstica pequeña. Los títulos decrecieron en los días subsecuentes hasta niveles de $10^{2.3}$ DIE_{50}/mosca para la mosca doméstica y $\geq$1DIE_{50}/mosca para la mosca doméstica pequeña al quinto día posterior a la infección. Ambas especies de moscas adquirieron títulos virales mayores que la dosis infectiva para un ave susceptible ($10^{3.0}$ DIE_{50}–$10^{4.0}$ DIE_{50}). Adicionalmente, las aves infectadas con leche evaporada que contenía un título alto de virus velogénico de la enfermedad de Newcastle, mantuvieron títulos por encima de la dosis infectiva para aves por hasta cuatro días posteriores a la exposición al alimento infeccioso. Las moscas alimentadas en heces de aves mantuvieron la dosis infectiva por solo un día. La disminución progresiva en los títulos se explicó significativamente ($P < 0.05$) para ambas especies de moscas mediante una regresión logística. La curva de regresión fue igual para las dos especies de moscas ($P < 0.05$), indicando una tasa similar de eliminación del virus.

Key words: flies, Newcastle disease virus, poultry, chickens, mechanical vector

Abbreviations: AAF = amino-allantoic fluid; ECE = embryonated chicken eggs; EID = egg infectious dose; EID_{50} = 50% egg infectious dose; END = exotic Newcastle disease; ENDV = exotic Newcastle disease virus; HA = hemagglutination activity; NDV = Newcastle disease virus; SEPRL = Southeast Poultry Research Laboratory; SPF = specific pathogen free; TCV = turkey coronavirus

Exotic Newcastle disease (END) is a contagious and fatal viral disease affecting the respiratory, nervous, and digestive systems of poultry and other birds. There is a near-100% death rate in unvaccinated poultry, and infections with END virus (ENDV) (Family *Paramyxoviridae*, genus *Avulavirus*) can even cause death in poultry vaccinated against the endemic low-virulence Newcastle disease virus (NDV) strains (11,25).

Significant epizootics of exotic Newcastle disease have occurred in the United States. The first epizootic, in 1971–1973, resulted in the quarantine of eight California counties, the destruction of 11.9 million birds, and eradication costs of $56 million (24). The second, in 2002–03, was also predominantly in California and resulted in the quarantine of 18,345 premises, the destruction of 3.2 million birds, and eradication costs of $170 million (7).

The virus is primarily spread by direct contact between infected and healthy birds. However, it can also be transmitted indirectly via contaminated equipment and persons (8,26). Wet manure will support the survival of ENDV for up to 16 days following virus

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shedding by an infected bird (12). Many insects, especially flies, are commonly associated with poultry operations, where they develop in wet manure (4,17,22). Adult flies can acquire animal pathogens from their environment and then transmit these pathogens to otherwise healthy hosts (1,9,23).

During the 2002–2003 END epizootic in southern California, ENDV was isolated from adult house flies (*Musca domestica* L.), little house flies (*Fannia canicularis* L.), and blow flies (*Phaenicia cuprina* Wiedemann) that were captured in the vicinity of ENDV-infected chickens (10). Viral titers associated with these infected flies were <1 EID_{50}/fly, far less than the 10^3 to 10^4 EID_{50} needed to infect a susceptible chicken (2,14). However, the number of flies collected in this study was small, and the time since these flies had been in contact with infectious material was unknown (10); therefore, it remains unclear whether adult flies can acquire enough ENDV, during contact with an infectious substrate (e.g., animal manure), to cause infection in a chicken that might consume the fly.

This study was conducted to determine the ability of house flies and little house flies to acquire and retain ENDV following exposure to an infectious food source; and to determine if virus persisted within the gut or outside the gut of the fly.

MATERIALS AND METHODS

The *Musca domestica* and *Fannia canicularis* used in this study were obtained from laboratory colonies established in 2005 using wild flies collected from several sites in southern California. The laboratory colonies were maintained at the University of California at Riverside, with larvae fed a standard medium (18). Approximately 500 pupae of each fly species were shipped separately on wet ice to the USDA Southeast Poultry Research Laboratory (SEPRL), where they were allowed to emerge over 48 hr into species-specific mesh net cages; they were provided with water and a standard diet of nonfat dry milk and sugar. After 48 hr, all pupae were removed from the cages to prevent further adult emergence. Flies were held at SEPRL in a biosafety level 3 agriculture facility at 25.5 C and 30% humidity.

A strain of ENDV (APMV-1/chicken/California/S0212430/02) isolated from backyard chickens in California during the 2002 END epizootic was used in this study. The virus was propagated by inoculation of 9- or 10-day-old specific pathogen free (SPF) embryonated chicken eggs (ECE) from the SEPRL white leghorn flock. Titration of the virus stock, infectious food, and fly homogenates was completed by preparing 10-fold serial dilutions of the allantoic fluid or sample supernate in brain heart infusion broth (BHI) with antibiotics; 200 µg gentamicin/ml, 2000 units penicillin/ml, and 4 µg amphotericin B/ml (Sigma Chemical Co., St. Louis, MO); 0.1 ml of each dilution was inoculated into five SPF eggs. Virus titer was determined by calculation of the 50% egg infectious dose (EID_{50}).

Experimental design. Following adult fly emergence, 2- to 3-day-old flies were starved for 24 hr prior to being given a sterile Petri-dish containing 15 ml of allantoic fluid containing a high titer of ENDV mixed with either 30 ml of evaporated milk or 30 g of fresh poultry feces collected from SPF chickens. The infectious poultry feces were fed only to house flies, while the infectious evaporated milk was fed to both fly species. The virus titer of the evaporated milk mixture was determined, using the methods described above, to be $10^{8.3}$ EID_{50}/0.1ml. In contrast to the evaporated milk mixture, poultry feces mixed with ENDV showed variation in titer across samples tested, perhaps due to incomplete mixing or to virus adsorbed to fecal material. Two samples of the poultry manure mixture were titered, giving virus concentrations of $10^{6.5}$ EID_{50}/0.1 g and $10^{5.1}$ EID_{50}/0.1 g, for a mean titer of $10^{5.8}$ EID_{50}/0.1 g. The reduced virus titer in the poultry feces, as compared to the titer in evaporated milk, may have been due to virus adsorbed to fecal material being pelleted by centrifugation prior to removal of the supernate for titration; or perhaps to inactivation of virus caused by environmental factors associated with fresh poultry feces, such as pH or the presence of microorganisms.

Flies were allowed to feed on either the infectious milk or infectious feces for 24 hr, after which the Petri-dish was removed and replaced with water and a standard diet of non-fat dry milk and sugar. After the 24-hr exposure period, the virus titer of the evaporated milk containing ENDV was determined to be $10^{7.9}$ EID_{50}/0.1ml, while the titer of the poultry feces containing ENDV was not measured. House flies starved for 24 hr will consume approximately 4 or 5 µl of evaporated milk for male and female house flies, respectively, within the first couple of minutes (unpublished data). Following the exposure period, ten flies were removed from the cages by sterile mechanical aspirator, every 24 hr, through day 5 for house flies or day 8 for little house flies. Flies were placed in a freezer at −20 C for 10 min to kill them, and were then separated into two groups of five flies of mixed sex, for each collection day, fly species, and infectious food source. Nonelectrical components of the mechanical aspirator were submerged in a disinfecting solution containing 1-Stroke Environ® (STERIS Corporation, St. Louis, MO), prepared following the manufacturer's directions and then rinsed following each use. The aspirator handle, which contained the battery, was wiped down with the same disinfectant solution.

One group of five flies was surface sterilized by immersion in 10% bleach for 30 sec, followed by a dip in 1 ml of BHI, to remove any residual bleach which might interfere with the virus isolation technique. Flies were pinned into a sterile wax dissecting dish with their ventral surface facing up. The mid- and hindgut (collectively the 'gut') was revealed by a mid-ventral incision from the head to the terminal abdominal plate and then the gut, distal to the crop, was excised from the rest of the fly body using sterile dissecting instruments. Dissecting dishes were wiped down with 1-Stroke Environ®, followed by BHI, and dissecting instruments were sterilized by placing them for 15 sec in a glass-bead sterilizer, heated to a temperature of 250 C before each dissection. The excised gut from each fly within a group was pooled into a 1.5 ml microcentrifuge tube (gut pool), and the remainder of each fly body was placed into a separate 1.5 ml microcentrifuge tube (rest of body pool). Flies in the second group were neither surface sterilized nor dissected prior to being pooled into a 1.5 ml microcentrifuge tube (whole body pool). All microcentrifuge tubes contained 1.5 ml of BHI with antibiotics; 200 µg gentamicin/ml, 2000 units penicillin/ml, and 4 µg amphotericin B/ml (Sigma Chemical Co.), and were stored at 4 C.

Fly pools were homogenized using a tissue grinder (Kontes Chemistry and Life Science Products, Vineland, NJ) with sterile plastic pestles. The fly homogenate was then centrifuged (Microfuge, Eppendorf North America, Westbury, NY) at $16{,}000 \times g$ for 10 min, and the supernatant was transferred to another sterile 1.5 ml microcentrifuge tube. Virus isolation was performed by inoculating 100 µl of the supernatant into the allantois of each of three 9- or 10-day-old SPF eggs. Eggs were incubated at 37 C in a standard humidified incubator. Eggs were candled to determine embryo death each 24 hr through 7 days postinoculation. Embryos that died within the first 24 hr were discarded. Embryos that died between 24 hr and 7 days, as well as all survivors at 7 days, were chilled at 4 C. Amnio-allantoic fluid (AAF) harvested from chilled eggs was tested for hemagglutination activity (HA) to detect ENDV. Virus presence in HA-positive samples was confirmed by hemagglutination-inhibition (HI) with NDV-specific antiserum (13). Amnio-allantoic fluids from HA-negative dead embryos, and embryos alive at 7 days postinoculation, were subjected to a second serial passage by inoculation of 100 µl of the AAF into each of three additional embryonated chicken eggs. Eggs were candled and killed embryos were handled as before. If, by day 7 postinoculation there was no HA activity in the AAF of the second passage dead or surviving embryos, the specimen was regarded as negative for ENDV.

For both fly species, virus titer was determined for one fly pool from each collection date, infectious food source, and pool type by the methods described above to titer the stock virus. Virus titers were log

Table 1. ENDV titers for pooled whole flies (W), pooled mid- and hindguts (G), and pooled fly bodies after excision of the gut (R) following a 24-hr exposure period to evaporated milk containing $10^{8.3}$ EID_{50}/0.1 ml or poultry feces$10^{5.8}$ EID_{50}/0.1 g.

	Evaporated milk[A]						Poultry feces[B]		
	House fly			Little house fly			House fly		
Days	W	G	R	W	G	R	W	G	R
1	5.69[C]	5.98	2.17	1.98	4.78	1.18	4	3.4	++
2	4.58	4.62	2.78	3.98	3.98	2.18	1.6	++	+
3	3.58	3.58	2.17	3.58	3.38	++	+	++	+
4	3.18	2.98	2.18	1.78	3.18	++	++	+	+
5	2.38	2.38	2.18	++	++	++	+	–	+
6	NT	NT	NT	1.18	++	–	NT	NT	NT
7	NT	NT	NT	–	+	+	NT	NT	NT
8	NT	NT	NT	–	+	–	NT	NT	NT

[A]For both fly species, viral titers were similar for the gut and whole body pools, which were significantly higher than the viral titer in the rest of the body pools ($P < 0.05$).

[B]House flies fed on infective poultry feces were not analyzed for differences in ENDV titer by pool type.

[C]Sample virus titer: $\log_{10}$ EID_{50}/fly or (++) = virus positive in the first passage of sample with a titer of ≥1 EID_{50} per fly; (+) = virus positive in second passage of sample with a titer of <1 EID_{50} per fly; (–) = no virus detected; (NT) = not tested.

transformed and compared by pool type (treatment) for each fly species using a repeated measures ANOVA with a Tukey-Kramer multiple comparison test to separate treatment means. A significant difference was indicated by $P < 0.05$. Fly pool types that were not significantly different were combined for further analysis. For house flies and little house flies fed evaporated milk with ENDV, virus titers from 1–5 day postexposure were compared using a Wilcoxon matched-pairs sign-ranks test. For both fly species, virus decay over time was examined by logistic regression analysis, with the slope of the regression lines compared by *F*-test to determine if the virus decay rate differed by fly species. House flies fed infective poultry feces were not analyzed for virus titer differences, by pool type or over time, due to low titers at 48 hr and later postexposure.

RESULTS

Flies fed evaporated milk with a high titer of ENDV ($10^{8.3}$ EID_{50}/0.1 ml) retained infectious virus through day 5 and day 8 postexposure for house fly and little house fly, respectively, after which virus isolations were no longer performed (Table 1). Similarly, house flies fed poultry feces with ENDV ($10^{5.8}$ EID_{50}/0.1 g) also retained infectious virus for up to 5 days postexposure. Virus titers decreased from day 1 postinoculation through day 5 postinoculation for whole body and excised gut pools. The low day 1 postinoculation titer for the little house fly whole body pool was thus considered an outlier and was removed from further analysis.

For both house flies and little house flies, viral titers were similar for the gut and whole body pools, which were significantly higher than the viral titer in the rest of the body pools ($P < 0.05$). For flies fed evaporated milk containing ENDV, house flies had a significantly higher virus titer than little house flies over the 1–5 day postexposure period ($W = 24$, $P = 0.04$), and both fly species maintained virus titers above the minimum infective dose for a chicken (10^3 EID_{50}–10^4 EID_{50}) for up to 4 days postexposure. House flies fed infectious poultry feces had a significantly lower virus titer relative to house flies fed infectious evaporated milk over the 1–5 day postexposure period ($W = 21$, $P = 0.01$), but virus titers were still above the minimum infective dose for a chicken for up to 24 hr postexposure.

There was a significant relationship between the number of days postexposure to evaporated milk containing ENDV and the virus titer for the gut and whole body pools, with titers decreasing from nearly 10^6 EID_{50}/fly on day 1 postexposure to 10^2 EID_{50}/fly on day 5 postexposure in house flies; and from nearly 10^5 EID_{50}/fly on day 1 postexposure to ≥1 EID_{50}/fly on day 5 postexposure in little house flies (Fig. 1). Regression equations for house fly ($y = -0.84x + 6.42$; $R^2 = 0.97$; $P < 0.001$) and little house fly ($y = -0.88x + 6.64$; $R^2 = 0.86$; $P < 0.001$) gave expected *x*-intercept values (time to complete loss of virus) of 7.64 and 7.5 day, respectively. This is in agreement with the very low virus titers determined for little house fly on day 7–8 postexposure, where virus was either not detected in fly pools or was only detected following second serial passage of the AAF of surviving embryos. The slope of the regression line was not different between fly species ($F = 0.07$, $P = 0.79$), indicating a similar rate of virus loss.

DISCUSSION

Avian paramyxoviruses, including ENDV, are most consistently isolated from infected poultry in feces and cloacal swabs (2). However, the maximum titer of ENDV shed in the feces of infected chickens has not been determined. Alexander *et al.* (3) found ENDV titers in feces of chickens infected with an ENDV isolate from a

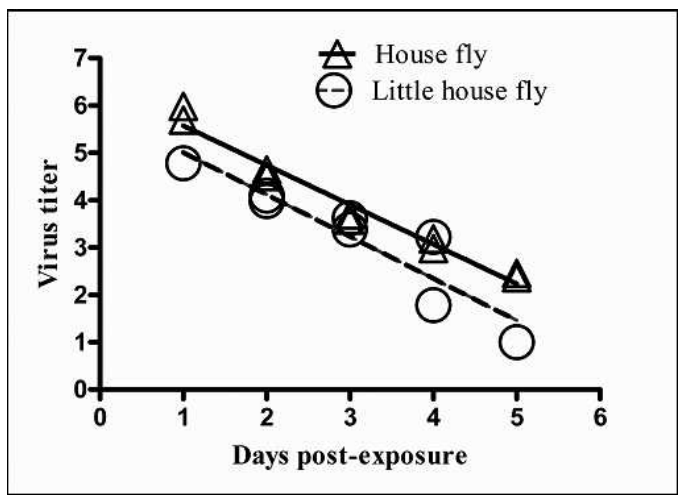

Fig. 1. Rate of decay of ENDV titer ($\log_{10}$ EID_{50}/fly) for house fly ($y = -0.84x + 6.42$; $R^2 = 0.97$; $P < 0.001$) and little house fly ($y = -0.88x + 6.64$; $R^2 = 0.86$; $P < 0.001$).

1933 outbreak in England (strain Herts 33/56) to be 10^5 EID_{50}/0.1 g feces at day 4 postinfection. However, viral titers were not determined in the feces beyond day 4 postinfection, and titers may have increased in subsequent days. Kapczynski and King (11) recovered a maximum ENDV titer of $10^{6.6}$ EID_{50} per cloacal swab from chickens infected with a California ENDV isolate. An avian influenza virus has been isolated from the feces of muscovy ducks at a titer of $10^{8.8}$ EID_{50}/0.1 g feces (28). Substantial virus concentrations would be expected in the feces of infected poultry, with virus titers perhaps as high as the virus titer of $10^{8.3}$ EID_{50}/0.1 ml evaporated milk and almost certainly as high as the mean virus titer of $10^{5.8}$ EID_{50}/0.1 g of poultry feces provided as food to flies in this study.

Starved flies that were provided evaporated milk containing a high titer of ENDV fed to repletion on the infectious meal and became transiently infected with ENDV at high concentration. With an average meal size for both sexes of house fly of 4.5 μl, and a mean virus titer of $10^{8.1}$ EID_{50} /0.1 ml for the infectious food source over the 24 hr exposure period, a fly would be expected to consume approximately $10^{6.8}$ EID_{50} of virus during a single feeding. At 24 hr postexposure, house flies in this study carried a mean virus titer of nearly $10^{6.0}$ EID_{50}/fly and little house flies carried a mean virus titer of $10^{4.8}$ EID_{50}/fly. Given the larger body size of the house fly relative to the little house fly, it is likely that the higher virus titer in house flies is due to greater meal size.

Starved flies, provided with poultry feces containing the same volume of high titer ENDV, acquired much less virus after the 24-hr exposure period relative to flies fed infectious evaporated milk, perhaps due to reduced feeding, inaccessibility of virus to feeding flies, or inactivation of the virus in the poultry feces. At 24 hr postexposure, house flies fed infectious poultry feces carried a mean virus titer of $10^{3.7}$ EID_{50}/fly.

Virus was detected through day 5 and day 8 postexposure for house flies and little house flies, after which virus isolation was no longer performed. Similarly, Rogoff *et al.* (21) fed little house flies with chorioallantoic fluid containing an ENDV isolate obtained during the 1971–73 ENDV outbreak and detected virus in these flies for up to 6 days, after which they were no longer tested. Additionally, Milushev *et al.* (19) recovered NDV from the body surface of house flies for up to 96 hr, and from fly homogenates for up to 10 days, when house flies were infected with an unknown strain and concentration of virus. The minimum infective dose of ENDV for a chicken has been shown to be 10^3–10^4 EID_{50} (3,14). In this study, both fly species exposed to high concentrations of ENDV in evaporated milk maintained virus at titers above the minimum infective dose for a chicken for up to 4 days after the period of exposure. Flies fed a high concentration of ENDV in poultry feces maintained virus titers above the minimum infective dose for a chicken for up to 24 hr after the period of exposure. Given the decreasing virus titer associated with both fly species over time, the concentration of virus initially consumed by a fly will determine if, and for how long, the virus titer in the fly will be above the minimum infective dose threshold for a susceptible chicken. Neither Rogoff *et al.* (21) nor Mulishev *et al.* (19) determined the titer of virus from fly pools following the period of exposure, so it is unknown how long flies in these studies might have retained virus at concentrations above those needed to infect a susceptible chicken. Rogoff *et al.* (21) were able to demonstrate virus transmission by little house flies fed to susceptible chickens for up to 48 hr following exposure of the flies to an infectious food source containing high titer ENDV and 5% sucrose. In contrast, Watson *et al.* (27) found that house flies infected in the laboratory, with a similar titer of a mesogenic form of the virus (NDV Roakin), held virus for a much shorter period of time, with virus titers falling to very low levels after only 9 hr. It was suggested by Watson *et al.* (27) that proteolytic enzymes within the fly midgut might be rapidly inactivating the virus. However, in this study virus persistence at moderate to high titer for several days suggests that midgut proteolytic enzymes may be less efficient at inactivating the ENDV strain used in this study. The virus strain used in this study (APMV-1/chicken/California/S0212430/02) is a field-derived velogenic strain of ENDV, while the NDV Roakin strain used by Watson *et al.* (27) is a less virulent, mesogenic vaccine strain. Newcastle disease virus strains are known to vary significantly in virulence and persistence in avian hosts (12), and differences in persistence within infected flies may be similarly expected.

Virus persistence was predominantly in the mid- and hindgut, as indicated by similar virus titers for the gut and whole body pools of both house flies and little house flies. Although flies landed on the infectious food source to initiate feeding, and would have been expected to contaminate portions of their legs and mouthparts, the virus titer associated with the fly body following excision of the mid- and hindgut, was significantly lower than the titer associated with the excised gut. The isolation of low titers of ENDV from fly tissues other than the excised gut may be due to the retention of virus within the crop or mouthparts of the fly; to passage of the virus across the gut barrier; or perhaps due to limited contamination of the fly body during the dissection and removal of the gut. Watson *et al.* (27) demonstrated some retention of the mesogenic NDV Roakin strain for up to 96 hr in the house fly crop, following feeding on an infectious food source.

The viral titer associated with both the house fly and the little house fly was significantly related to the time-since-exposure to the virus, with log transformed titers decreasing linearly with time. Watson *et al.* (27) also showed that virus titer was significantly related to time, but virus decay was best described by a logistic regression of log transformed virus titers. Overall, the virus decay of ENDV in this study was considerably slower than the decay rate of NDV Roakin shown by Watson *et al.* (27), where virus titers were <1 ID_{50} at 9 hr postexposure and not detectable in the gut by 96 hr postexposure.

This study provides further evidence that flies may serve as efficient vectors of animal-associated enteric pathogens. Shane *et al.* (23) demonstrated the potential for house flies to transmit pathogens by placing flies into cages with *Campylobacter jejuni*-infected chickens and subsequently moving the flies to cages with pathogen free chickens, resulting in *C. jejuni* infection in the previously uninfected chickens. Similarly, turkey poults housed in isolation units became infected with turkey coronavirus (TCV) when house flies orally inoculated with TCV were released into the isolation units for 24 hr (9), and cattle held in confined pens became infected with *Escherichia coli* O157:H7 after house flies orally inoculated with the bacteria were released into the pens for 48 hr (1). In addition to the demonstration of virus transmission by orally inoculated little house flies to susceptible chickens, Rogoff *et al.* (21) also conducted preliminary testing to determine if flies were capable of acquiring ENDV from infected chickens and subsequently transmitting the virus to susceptible chickens. While they did demonstrate virus transmission between infected and susceptible birds in test enclosures connected by open tubes to allow for fly movement, it was not clear that virus transfer was due to the movement of flies, as they suggest, or due to movement of aerosolized virus through the open tubes.

Flies are known to acquire ENDV under natural field settings (10). These studies have shown that both fly species, when exposed

to a food source with a high concentration of ENDV, can retain virus for several days at titers greater than that required to infect a susceptible chicken. Flies, especially house fly and little house fly, are commonly associated with poultry operations and are well known to disperse into surrounding areas (4,5,17). With an average lifespan for an adult house fly of 3–7 days (15,16), and the ability to disperse up to 9.6 km within 24 hr (6,20), the opportunity for dispersal of ENDV from one infected poultry facility to another may be substantial. This study highlights the importance of fly control measures as part of a general biosecurity plan, especially during outbreaks of END and other avian virus diseases. More research is needed to examine the actual role of flies and other insects as mechanical vectors of ENDV in field settings. Dispersal and survival of infected flies should be examined, and mathematical models should be developed to describe the interaction of ENDV, vector insects, and susceptible chickens.

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